



Can artisanal “Coalho” cheese from Northeastern Brazil be used as a functional food?

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ARTICLE INFO

Article history:

Received 19 January 2012

Received in revised form 1 June 2012

Accepted 14 June 2012

Available online 29 June 2012

Keywords:

Artisanal “Coalho” cheese

Functional food

Bioactive peptides

Cheese

ABSTRACT

Brazilian artisanal “Coalho” cheeses from six Northeast towns were investigated as a functional food based on their peptide profiles and antioxidant, zinc-binding and antimicrobial activities. The peptides (WSP) from “Coalho” cheese showed high antioxidant activity, the best value of TEAC being $2223 \pm 10.10 \mu\text{M}$, which means $91.1 \pm 0.43\%$ oxidative inhibition and peptide concentration for IC_{50} of 7 mg/mL ($21 \mu\text{g}$ of peptides) for sample from the town of Correntes. The smallest TEAC value ($1896 \pm 17 \mu\text{M}$), which means $75.9 \pm 0.7\%$ oxidative inhibition and IC_{50} of 10.5 mg/mL ($31.5 \mu\text{g}$ of peptide), was obtained for samples from the town of São Bento do Una. The zinc-binding activities were: Arcoverde ($72.21 \pm 0.24\%$) Cachoeirinha ($75.02 \pm 0.02\%$), Capoeiras ($61.78 \pm 0.65\%$), Correntes ($75.47 \pm 0.5\%$), São Bento do Una ($75.41 \pm 0.15\%$), and Venturosa ($74.36 \pm 0.04\%$). The WSP extracts showed antimicrobial activity against *Enterococcus faecalis*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*. All the results obtained suggest that “Coalho” cheese has potential as a functional food.

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1. Introduction

The term “functional foods”, closely related to health maintenance and preventive medical care, was first introduced in Japan during the 1980s when the government financed a national research project on the implications of medical sciences for diet, in order to guarantee good health conditions for the older population (Arias-Aranda & Romerosa-Martínez, 2010). Most experts agree on the following definition: “A food can be regarded as functional if it is satisfactorily demonstrated to affect beneficially one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either improved state of health and well-being and/or reduction of disease risk”, included in the EC Project FUFOSSE Consensus Document of 1999 (Arias-Aranda & Romerosa-Martínez, 2010).

The artisanal “Coalho” cheese is a Brazilian product typically Northeastern and very popular, widely consumed by the local

population and around Brazil. The main features of this cheese are its slightly salty and acid flavour, and resistance to heat without melting allowing the preparation of the popular “roast cheese”. The “Coalho” cheese has been produced primarily in the Northeastern States of Brazil: Pernambuco, Ceará, Rio Grande do Norte and Paraíba. This cheese has considerable input in the economy, being significant in the income of milk suppliers, especially those who do not have access to milk processing plants.

Over the past two decades, studies have been focused on the biological properties of milk proteins (80% casein), which possess additional physiological effects due to the numerous bioactive peptides that are encrypted within intact proteins (Korhonen & Pihlanto, 2006). During cheese manufacturing and ripening, proteinases from diverse origins degrade caseins ($\alpha\text{s}1$ -, $\alpha\text{s}2$ - β and κ) releasing peptides of different sizes. These peptides, once released, exhibit different bioactivities on the digestive, cardiovascular, immune and nervous systems (Foltz, Van Buren, Klaffke, & Duchateau, 2009; Korhonen & Pihlanto, 2006).

Based on the health and biotechnological potentials of the Artisanal “Coalho” cheese above described, the aim of the present study was to investigate its bioactivity as an antioxidant, its zinc-binding and antimicrobial properties, to examine its potential as a functional food.

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2. Materials and methods

2.1. Chemical and reagents

All chemicals and reagents were of analytical grade purchased from Merck KGaA (Darmstadt, Germany), Sigma–Aldrich Chemie GmbH (Steinheim, Germany) and Biotium (Hayward, CA).

2.2. Materials

Samples of artisanal “Coalho” cheeses were collected directly from producers in the following towns of Agreste Region of Pernambuco State, Brazil: Arcoverde, Capoeiras, Cachoeirinha, Correntes, São Bento do Una and Venturosa. The samples were collected in sterile plastic bags, kept at 10 °C on their journey to the laboratory on the same day and kept at –20 °C until analysis. One cheese was collected from each town and one producer according to the police agency of Pernambuco State Farming. After three months the process was repeated for new samples. The production process of artisanal “Coalho” cheese, using animal industrial rennet (chymosin), is performed according to the flowchart in Fig. 1.

The microorganisms used for antimicrobial activity (*Enterococcus faecalis* ATCC 6057, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 29665, and *Staphylococcus aureus* ATCC 6538) were obtained from the Department of Mycology – UFPE.

2.3. Extraction of water-soluble peptides (WSP)

Each cheese sample was homogenized with water (1:2 w/v) at 1000 rpm for 5 min in a Nissei AM-8 homogeniser, followed by

centrifugation at 8000g for 30 min at 4 °C. The supernatant containing the water-soluble peptides (WSP) was collected and the precipitate discarded. The centrifugation process was repeated twice, using the same conditions, in order to obtain a supernatant free of precipitate. The clear WSP extract was freeze-dried and stored at –20 °C.

2.4. Peptide determination of the crude WSP extracts

Peptide concentrations were measured by Folin-phenol method (Lowry, Rosebrough, Farr, & Randall, 1951) using bovine serum albumin as standard.

2.5. Peptide profile of WSP in MALDI-ToF mass spectrometry analysis

The peptide profiles of the WSP extracts obtained from “Coalho” cheese samples were measured using an Ettan MALDI-ToF-Pro (Amersham Biosciences, Sunnyvale, CA) equipped with a quadratic reflectron field and timed ion gate. The acquisition of intact peptides was performed in linear mode with positive ionisation, rejection of mass 500 *m/z*, velocity of 8 shots/s, ion accelerating potential of 20 kV. An average of 256 shots were collected for each spectrum. Each WSP sample was purified using C18 Zip Tips and 150 µL of 0.1% (v/v) trifluoroacetic acid. An aliquot of 1 µL from each mixture was mixed with matrix solution 4-HCCA (α-cyano-4-hydroxycinnamic acid in 50% v/v acetonitrile containing 0.1% v/v trifluoroacetic acid) in the proportion of 1:1 (v/v). Aliquots (0.3 µL) from the last mixture were applied to four different spots on a sample slide tray, dried at room temperature (23–25 °C) and inserted into the mass spectrometer to obtain the spectra. Calibration of the time-to-mass scale was performed using two external standard peptides (Ile7AngIII, bradykinin M+H 897.531, monoisotopic, and hACTH 18–39, M+H 2465.191, monoisotopic).

2.6. Antioxidant activity of WSP samples using 2,2-azino-bis-(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS)

According to Re et al. (1999), the ABTS assay is based on the generation of chromophore cationic radical (ABTS⁺) obtained from the oxidation of ABTS by potassium persulfate. The oxidation reaction was prepared with 7 mM ABTS stock solution with 140 mM potassium persulfate (final concentration), the mixture was left in the dark at room temperature (23–25 °C) for 12–16 h (time required for radical formation) before its use. The ABTS⁺ solution was diluted in ethanol to an absorbance of 0.7 (±0.02) units at 734 nm. The effect of WSP amount on the antioxidant activity was carried out using aliquots of 30 µL, containing 3.5, 7.0, 10.5, 14.0 or 17.5 mg peptides/mL, and mixing with 3 mL diluted ABTS⁺ solution. The absorbances at 734 nm were measured at different time intervals (0, 6, 30, 60, 90, 150 and 180 min). Appropriate solvent blanks were run in each assay. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as a reference standard. The values of oxidative inhibition percentage were calculated and plotted as a function of the reference antioxidant concentration (Trolox) and expressed as Trolox equivalent antioxidant capacity (TEAC, µM). All determinations were carried out in triplicate.

2.7. Zinc-binding activity of “Coalho” cheese WSP extracts

Solution of 1 mg/mL zinc chloride (ZnCl₂), prepared in sodium phosphate buffer (100 mM; pH 7.0), was added to each WSP sample (30 mg peptides/mL) and incubated for 60 min at 36 °C, according to Dashper et al. (2005). After this incubation, each sample was dried at 105 °C for 24 h and digested to measure the

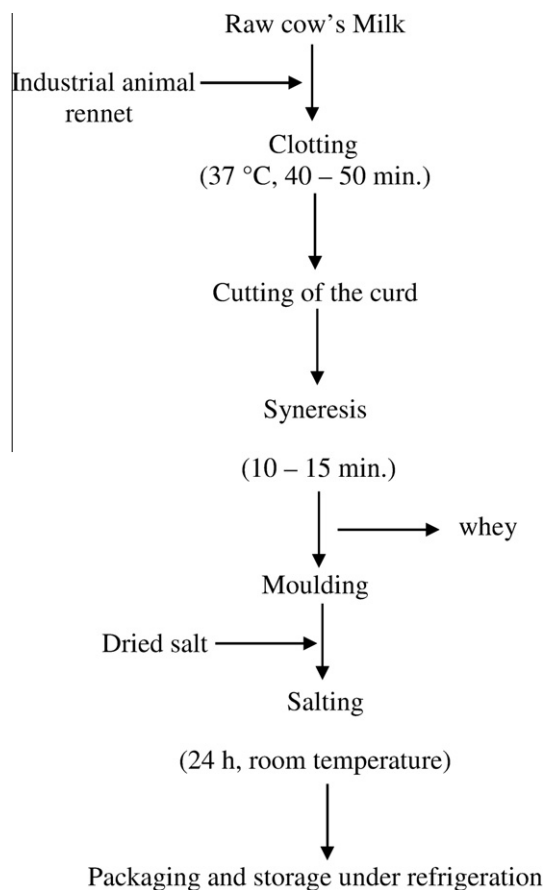


Fig. 1. Production flowchart of Brazilian Northeast artisanal “Coalho” cheese.

amount of zinc bound to peptides, as recommended by the Association of Official Agricultural Chemists (AOAC., 2000).

Samples (100 mg) of dried WSP were ashed in a muffle furnace (500 °C) for 3 h, until a constant weight was obtained. The concentration of zinc in the white ash from each WSP sample was measured using inductively coupled plasma-optical emission spectrometry (ICP-OES) at 213.9 nm. The zinc-binding activity was expressed based on the percentage of zinc bound to the peptides contained in the WSP extract from “Coalho” cheese, calculated using the initial and final zinc concentrations compared to a control without peptides.

2.8. Determination of antimicrobial activity

Each dried WSP sample was prepared in sterile distilled water at final concentration of 50 mg/mL, centrifuged at 1000g for 10 min and the supernatant used for the antimicrobial activity assay.

The microorganisms were selected according to the National Committees for Clinical Laboratory Standards (NCCLS, 2003). Gram positive bacteria: *S. aureus* ATCC 6538, *B. subtilis* ATCC 6633, *E. faecalis* ATCC 6057 and Gram negative bacteria such as *P. aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 29665 and *E. coli* ATCC 25922 were used. Pre-inoculum for each standard strain was prepared in TSB (tryptic soy broth; Acumedia, Baltimore, MD) and incubated at 37 °C for 18–24 h. Each inoculum was prepared according to McFarland standard for 1.5×10^8 cfu/mL. All experiments were carried out in a 96-well plate (Nunc), where each well received the standard strain inoculum, liquid culture medium broth TSB and WSP samples for a final volume of 100 μ L (NCCLS, 2003). A WSP control was only composed by peptide sample and culture medium. The microplate was then incubated at 37 °C for 18–24 h. The detection of antimicrobial activity was assessed by cell viability using a commercial kit (Resazurin Cell Viability Assay Kit, Biotium Inc.). After the incubation period, 30 μ L resazurin solution were added to each well (Palomino et al., 2002), reincubated for 30 min and analysed by staining. A pink colour or lack of colour indicates growth of bacteria and the purple or blue colour the inhibition of growth.

2.9. Statistics analysis

The statistical significance of all experimental data was carried out by software Statistica 8 using parametric tests. One-way analysis of variance (ANOVA) was applied to determine the difference among the groups, followed by Tukey post hoc test. Differences were considered significant at $p < 0.05$.

3. Results and discussion

Proteolysis is the most complex of all the primary events during the ripening of cheeses, which results in the formation of various peptides. These peptides not only contribute towards the development of flavour and texture in the ripened cheeses but also show a substantial bioactivity (Saito, Nakamura, Kitazawa, Kawai, & Itoh, 2000). Proteolysis also can occur during the production of fresh cheeses such as artisanal “Coalho” cheese, resulting in a number of peptides, as shown in Table 1.

The peptides are the main agents responsible for the bioactivity of this Brazilian cheese and their molecular weights range from 800 to 3500 Da. The number of different peptides present in the cheese from each town was: Arcoverde – 67, Capoeiras – 57, Cachoeirinha – 70, Correntes – 71, São Bento do Una – 72 and Venturosa – 57. Some of these peptides have been identified in cheeses such

as Cheddar, Swiss, Edam, Cooleeney, Camembert, Parmigiano-Reggiano, Port Salut, and Gruyere (Piraino et al., 2007).

3.1. Antioxidant activity (ABTS⁺ radical) of WSP from Artisanal “Coalho” cheeses

Fig. 2 shows that all WSP extracts (17.5 mg peptides/mL) from artisanal “Coalho” cheeses had antioxidant activity as a function of time. WSP extract from Correntes had the greatest antioxidant capacity with $91.1 \pm 0.43\%$ oxidative inhibition after 180 min, equivalent to TEAC of $2221 \pm 10.18 \mu\text{M}$ Trolox. The Tukey post hoc test showed that this result was different when compared to those obtained from other towns: Cachoeirinha ($85.91 \pm 0.88\%$; $p = 0.0006$); São Bento do Una ($77.92 \pm 0.70\%$; $p = 0.0001$); Arcoverde ($84.19 \pm 0.70\%$; $p = 0.0007$); Capoeiras ($87.77 \pm 1.69\%$; $p = 0.0004$) and Venturosa ($82.84 \pm 2.57\%$, $p = 0.0002$). While the “Coalho” cheese from São Bento do Una town showed the lowest activity ($75.92 \pm 0.7\%$) after 180 min or TEAC of $1895.6 \pm 17.6 \mu\text{M}$ Trolox, significantly different from the other cheeses. Cheeses from Arcoverde, Cachoeirinha, Capoeiras and Venturosa did not present significant differences.

In addition, all WSP extracts reached maximum antioxidant activity after 90 min of incubation ($76.48 \pm 6.48\%$ or TEAC of $1852 \pm 141 \mu\text{M}$ Trolox); from 90 to 180 min there was an average increase of $8.37 \pm 2.6\%$ which is not statistically significant.

Fig. 3 shows the effect of peptide concentration on the ABTS⁺ scavenging activity. The highest values of ABTS⁺ scavenging activity, using 17.5 mg peptides/mL, for each cheese WSP sample were: Arcoverde ($76.27 \pm 0.55\%$); Cachoeirinha ($76.83 \pm 0.14\%$); Capoeiras ($73.2 \pm 0.14\%$); Correntes ($84.23 \pm 0.6\%$); São Bento do Una ($66.27 \pm 1.24\%$) and Venturosa ($75.1 \pm 1.98\%$), with TEAC values of 1868 ± 13.4 ; 1798 ± 4.37 ; 2052 ± 13.3 ; 1610 ± 30.0 ; $1827 \pm 49.5 \mu\text{M}$ Trolox, respectively. The results showed that the antioxidant activity was proportional to peptide amount for all sample studied. In this way the maximum value was obtained for the WSP extract from Correntes cheese, which was different to Arcoverde, Cachoeirinha, Capoeiras, São Bento do Una, and Venturosa (all $p = 0.0001$). The lowest antioxidant activity was obtained for cheese from São Bento do Una town which was different from all the other cheeses, while the other cheeses showed no statistically significant differences.

3.2. There were no significant differences in antioxidant activities in the peptide concentration range from 10.5 up to 17.5 mg peptides/mL

The peptide extracts from “Coalho” cheeses showed much better results than those obtained by Gupta, Mann, Kumar, and Sangwan (2009) for antioxidant activity of Cheddar cheese manufactured with adjunct cultures *Lactobacillus casei* ssp. *casei* 300 ($16.6 \mu\text{M}$ Trolox) and *Lactobacillus paracasei* ssp. *paracasei* 22 ($9.76 \mu\text{M}$ Trolox). According to Gupta et al. (2009) milk fermentation has been described as a strategy to release antioxidant peptides, capacity that some authors have attributed to the hydrolysed fractions from caseins. According to these authors, histidine and proline have been described as the most important amino acid residues responsible for the inhibition activity of peptides in lipo-protein peroxidation. Seven of the eight peptides identified in the highest antioxidant fraction contained at least one proline residue, and six of them had more than two proline residues. Tyrosine and tryptophan also showed ABTS⁺ radical scavenging capacity explained by the special characteristic of phenolic and indole groups to serve as hydrogen donors (Hernandez-Ledesma, Dávalos, Bartolome, & Amigo, 2005). The presence of leucine residues in the peptide sequence seems to play an important role in their antioxidant and ACE-inhibitory activities (Alemán, Giménez, Pérez-Santin, Gómez-Guillén, & Montero, 2011).

Table 1
The molecular weights (MW) of peptides formed during the manufacture of artisanal “Coalho” cheese according their mass spectrometry analyses (MALDI-ToF). Peptide molecular weight range from 800 to 3500 Da.

Arcoverde	Capoeiras	Cachoeirinha	Correntes	São Bento do Una	Venturosa
902, 999, 1024, 1052, 1098, 1143, 1151, 1172, 1209, 1253, 1269, 1360, 1383, 1399, 1421, 1468, 1493, 1511, 1568, 1597, 1609, 1623, 1649, 1676, 1708, 1726, 1762, 1790, 1862, 1881, 2000, 2053, 2064, 2081, 2119, 2167, 2247, 2263, 2319, 2343, 2363, 2391, 2456, 2479, 2531, 2635, 2651, 2764, 2779, 2846, 2929, 2955, 2968, 3008, 3062, 3069, 3107, 3137, 3146, 3182, 3270, 3331, 3385, 3418, 3484, 3499.	902, 909, 1052, 1096, 1151, 1208, 1246, 1253, 1269, 1360, 1385, 1399, 1437, 1468, 1493, 1511, 1568, 1597, 1609, 1624, 1623, 1649, 1676, 1708, 1726, 1790, 1881, 1904, 2000, 2018, 2119, 2166, 2263, 2343, 2363, 2409, 2456, 2479, 2523, 2536, 2635, 2778, 2843, 2890, 2929, 2954, 2985, 3043, 3069, 3104, 3156, 3183, 3269, 3383, 3479, 3493, 3500.	902, 910, 944, 960, 970, 1002, 1026, 1052, 1099, 1151, 1172, 1252, 1253, 1269, 1317, 1360, 1384, 1399, 1421, 1437, 1469, 1493, 1511, 1543, 1568, 1597, 1609, 1623, 1626, 1649, 1676, 1708, 1726, 1762, 1790, 1862, 1881, 2000, 2018, 2064, 2119, 2166, 2228, 2246, 2263, 2343, 2363, 2395, 2479, 2523, 2635, 2649, 2692, 2778, 2929, 2954, 2981, 3012, 3068, 3106, 3141, 3187, 3209, 3271, 3288, 3378, 3384, 3418, 3483, 3498.	902, 906, 944, 982, 1006, 1025, 1043, 1052, 1095, 1104, 1120, 1145, 1151, 1171, 1209, 1234, 1253, 1269, 1308, 1318, 1360, 1384, 1399, 1399, 1421, 1438, 1449, 1469, 1493, 1511, 1535, 1543, 1568, 1597, 1609, 1623, 1649, 1676, 1699, 1708, 1726, 1790, 1862, 1881, 2000, 2018, 2053, 2064, 2119, 2228, 2247, 2263, 2321, 2343, 2363, 2388, 2409, 2456, 2479, 2635, 2744, 2764, 2779, 2929, 2973, 3066, 3132, 3270, 3384, 3418, 3499.	902, 927, 970, 1005, 1027, 1052, 1099, 1115, 1144, 1151, 1190, 1202, 1244, 1253, 1269, 1317, 1338, 1360, 1383, 1399, 1421, 1467, 1493, 1511, 1568, 1597, 1609, 1623, 1676, 1708, 1725, 1745, 1753, 1790, 1845, 1881, 2000, 2018, 2035, 2064, 2080, 2119, 2166, 2263, 2331, 2343, 2363, 2395, 2444, 2479, 2522, 2563, 2635, 2778, 2843, 2888, 2929, 2970, 3015, 3043, 3068, 3097, 3134, 3185, 3207, 3269, 3288, 3337, 3383, 3417, 3482, 3498.	805, 906, 909, 1052, 1095, 1145, 1151, 1206, 1209, 1246, 1246, 1253, 1269, 1360, 1384, 1399, 1437, 1493, 1511, 1543, 1568, 1597, 1623, 1649, 1676, 1708, 1726, 1751, 1790, 1881, 1904, 2000, 2018, 2064, 2119, 2166, 2230, 2263, 2343, 2363, 2479, 2523, 2635, 2739, 2779, 2929, 2950, 2955, 3043, 3070, 3102, 3157, 3183, 3271, 3385, 3495, 3502.
Peptides total: 67	57	70	71	72	57

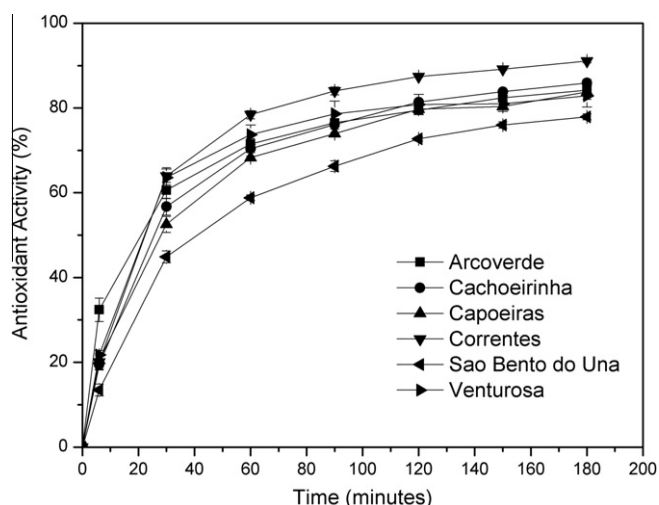


Fig. 2. Effect of the incubation time on antioxidant activity of WSP extracts from artisanal “Coalho” cheeses. WSP extracts at defined concentration of 17.5 mg/mL. Standard deviations were $\pm 2.0\%$ based on three replicates.

Free radicals scavenging activity was detected using an isolated 1000 Da peptide from peptic hydrolysate of casein that possessed a primary structure of Tyr-Phe-Tyr-Pro-Glu-Leu (Suetsuna, Ukeda, & Ochi, 2000). According to Pritchard, Phillips, and Kailasapathy (2010), fractions of peptide extracts from three commercial Australian Cheddar cheeses exhibited antimicrobial, antihypertensive and antioxidant properties. Moreover, Gupta et al. (2009) showed that the extent of antioxidant activity of these peptides was dependent on the ripening stage of the cheeses. It is noteworthy the antioxidant activity of the peptides not only depends on the amino acid composition but also on the sequence and configuration of the peptides (Chen, Muramoto, Yamauchi, Fujimoto, & Nokihara, 1998).

Casein peptides, molecular weight about 3000 Da, have been shown to possess strong antioxidant activity by the β -carotene bleaching method, and also showed scavenging activity against

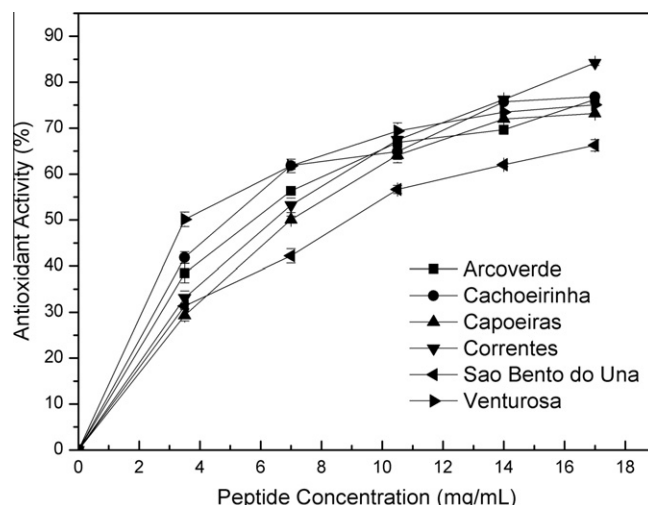


Fig. 3. Effect of peptide concentration in WSP extracts from artisanal “Coalho” cheeses on antioxidant activity at defined incubation time interval of 90 min. Standard deviations were $\pm 3.0\%$ based on three replicates.

free radicals superoxide, DPPH (2,2-diphenyl-1-picrylhydrazyl) and hydroxyl (Sakanaka, Tachibana, Ishihara, & Juneja, 2005).

Similar molecular weight peptides were identified in the WSP extracts from artisanal “Coalho” cheeses. These antioxidant peptides present in foods play a vital role in the maintenance of antioxidant defence systems by preventing the formation of free radicals or scavenging free radicals and active oxygen species, which induce oxidative damage to biomolecules and cause ageing, cancer, heart diseases, stroke and arteriosclerosis.

The results obtained for antioxidant activity of Artisanal “Coalho” cheeses were similar to those reported for some wines and antioxidant standards such as BHA (3-*tert*-butyl-4-hydroxyanisole) which possessed a TEAC of 2430 μM (Contreras, Hernández-Ledesma, Amigo, Martín-Álvarez, & Recio, 2011), twice as much as values for α -tocopherol and vitamin C (970 and 990 μM Trolox, respectively) obtained by Miller, Rice-Evans, Davies, Gopinathan, and Milner (1993).

In spite of the antioxidant activity versus reaction time, it was also observed that all WSP extracts reached IC_{50} after the first 30 min, except the sample from São Bento do Una town ($44.88 \pm 1.4\%$ oxidative inhibition) (Fig. 2). The results obtained on the effect of peptide concentration showed that IC_{50} for all cheese samples was reached with 21 μ g of peptide, which according to the assay conditions corresponded to 30 μ L aliquot from WSP sample containing 7 mg peptides/mL, except for cheese from São Bento do Una town ($42.2 \pm 1.57\%$).

3.3. Zinc-binding activity

All WSP extracts from artisanal “Coalho” cheeses studied showed zinc-binding activity greater than 50% (Fig. 4). The best binding percentage was detected for cheese from Correntes ($75.47 \pm 0.5\%$) and the lowest for cheese from Capoeiras ($61.78 \pm 0.65\%$). The value for Correntes cheese was not different from those obtained for Arcoverde ($72.21 \pm 0.24\%$) Cachoeirinha ($75.02 \pm 0.02\%$), São Bento do Una ($75.41 \pm 0.15\%$), and Venturosa ($74.36 \pm 0.04\%$), while the cheese sample from Capoeiras, which showed the lowest value, was not different from those obtained from Arcoverde, Cachoeirinhas and Venturosa, but the difference was significant compared with those obtained for cheeses from Correntes ($p = 0.033$) and São Bento do Una ($p = 0.026$).

These results are of great importance, because besides having other properties the “Coalho” cheese can increase the bioavailability of zinc in the body, since intestinal absorption of zinc is affected by a great number of dietary factors, which include proteins, calcium, and metal-complexing. Also, this mineral plays a key role in the function of several enzymes, participates in cell division, genetic expression, physiological processes like cellular growth, and development and genetic transcription (Salgueiro et al., 2000).

It has been reported that phosphorylated peptides encrypted in α s1-, α s2- and β -casein may form soluble complexes with minerals such as calcium, iron and zinc at intestinal pH, modulating their bioavailabilities. These peptides, which act as mineral solubilisers and/or carriers, are known as caseinophosphopeptides (CPPs) and can be released “in vitro” or “in vivo” by enzymatic digestion of dairy products or during their processing (Clare & Swaisgood, 2000; Meisel & FitzGerald, 2003). Many CPPs contain high polar acidic sequences of three phosphoserines followed by two glutamic acid residues (SpSpSpEE), which are the binding sites for minerals. Moreover, there is evidence that amino acid residues upstream and downstream of this region are also involved (Ferraretto, Gravaghi, Fiorilli, & Tettamanti, 2003).

Harzer and Kauer (1982), did not detect zinc binding to dephosphorylated casein; the conclusion might be drawn that the bivalent

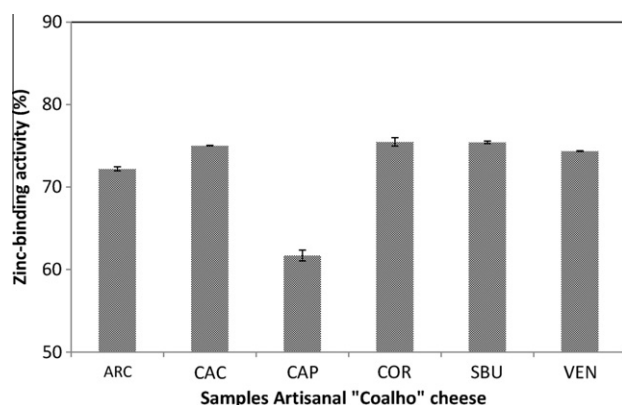


Fig. 4. Zinc-binding activities of WSP extracts from artisanal “Coalho” cheeses. Standard deviations were $\pm 0.65\%$ based on three replicates.

Table 2

Antimicrobial activities of WSP extracts from artisanal “Coalho” cheeses.

Strains	Antimicrobial activity					
	ARC	CAC	CAP	COR	SBU	VEN
<i>Enterococcus faecalis</i> ATCC 6057	+	+	+	–	+	+
<i>Bacillus subtilis</i> ATCC 6633	–	+	–	–	+	+
<i>Escherichia coli</i> ATCC 25922	–	+	–	–	–	+
<i>Pseudomonas aeruginosa</i> ATCC 27853	–	+	–	–	–	+
<i>Klebsiella pneumoniae</i> ATCC 29665	–	–	–	–	–	–
<i>Staphylococcus aureus</i> ATCC 6538	–	–	–	–	–	–

Legend of producer towns of Artisanal “Coalho” Cheese: Arcoverde (ARC); Capoeiras (CAP); Cachoeirinha (CAC); Correntes (COR); São Bento do Una (SBU); Venturosa (VEN).

zinc ion is complexed to casein by the negative charge of phosphate groups, which would be in good agreement with the fact that there was no zinc binding to casein at low pH, where the phosphate residues would be protonated.

Another important result about the zinc-binding activity of artisanal “Coalho” cheese was that the zinc bound weakly to phosphoserine residues in CPPs, and according to Sato, Noguchi, and Naito (1986), this weak affinity is relevant to nutrition, because zinc and other minerals can be released progressively in the intestinal lumen, allowing greater absorption of zinc.

3.4. Antimicrobial activity of artisanal “Coalho” cheeses

There are no previous reports about antimicrobial activity of “Coalho” cheese. The WSP extracts from artisanal “Coalho” cheeses showed antimicrobial activity for at least one standard strain tested, except for the WSP extract from Correntes that did not show antimicrobial activity (Table 2). The highest activities were obtained for cheeses from Cachoeirinha and Venturosa against *E. faecalis*, *B. subtilis*, *E. coli*, and *P. aeruginosa*.

López-Expósito, Gómez-Ruiz, Amigo, and Recio (2006) reported that the majority of peptides derived from casein with antimicrobial activity are in the range 3–50 amino acids, which are in the same molecular weight range found in this work (800–3500 Da). Some authors have reported that various peptides derived from milk casein have antimicrobial properties, such as casecidins obtained by chymosin digestion of α s1-casein which were intended for therapeutic use to treat infectious diseases. These peptides have bactericidal activity against a wide range of Gram-positive bacteria of health significance including staphylococci, *Sarcina* spp., *B. subtilis*, *Diplococcus pneumoniae* and *Streptococcus pyogenes* (Clare & Swaisgood, 2000). Isracidin is another antimicrobial peptide released by chymosin cleavage of bovine α s1-casein, which consists of a 23-amino acid-residue fragment called f(1–23). This cationic peptide has been reported to be active *in vitro* against a broad spectrum of Gram-positive and Gram-negative bacteria (Hayes, Ross, Fitzgerald, Hill, & Stanton, 2006). This type of peptide was also found within the known peptides contained in the WSP extracts from “Coalho” cheeses.

Recently, Pritchard et al. (2010) evaluated the antimicrobial activity of peptide extracts of Australian Cheddar cheeses and found activity against *E. coli* and *Bacillus cereus*. In addition, Italian cheese water-soluble peptides have shown high antimicrobial activity against various bacteria including *E. coli*, *Bacillus megaterium*, *Listeria innocua*, and *S. aureus* (Rizzello et al., 2005). Finally, the antimicrobial peptides from “Coalho” cheeses like other cheeses studied present the advantage of being derived from a harmless source, and may have therefore a great potential for use in preventive medicine or the food industry.

4. Conclusions

These findings showed that all water-soluble peptides (WSP) extracts from artisanal “Coalho” cheeses exhibited bioactivity. The peptides had high activities in all bioactive properties analysed. Although it has been difficult to compare the antioxidant capacity with the data from the literature due to the diversity of methodologies used, “Coalho” cheese seems to be a potential source of antioxidant peptides. The bioavailability of zinc in the body can be increased by the peptides from Brazilian cheese. The antimicrobial activity presented by WSP extracts can be an additional advantage during the production process, reducing possibly the contamination of milk foods and derivatives and increasing the shelf-life of the product. “Coalho” cheese peptides can represent a source of health-enhancing components that may be considered as functional foods or incorporated in pharmaceutical or nutraceutical preparations.

Acknowledgements

The authors thank the financial support of FACEPE (Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco), LIKA (Laboratório de Imunopatologia Keizo Asami), UFPE (Universidade Federal de Pernambuco) and UFRPE (Universidade Federal Rural de Pernambuco).

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